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## Laser Mikroskop

## Microscope laser

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- **PATENT ABSTRACTS OF JAPAN** vol. 2000, no. 01, 31 January 2000 (2000-01-31) & JP 11 271636 A (OLYMPUS OPTICAL CO LTD), 8 October 1999 (1999-10-08)

### Description

**[0001]** The present invention relates to a laser microscope for use particularly in biological, medical, and other applications, which irradiates a sample with a laser light constituted of a plurality of emission wavelengths through an objective lens and detects fluorescent light from the sample.

**[0002]** There is a laser microscope for use in biological, medical, and other applications. In the laser microscope, for example, it is requested to observe a live cell or tissue over a long time as it is.

**[0003]** For example, there is an observation of a change of a concentration of calcium in the cell or the tissue. A method of the observation comprises: dyeing a sample with a fluorescent indicator which emits a fluorescent light in accordance with the calcium concentration; irradiating the sample with a laser light (excitation light) of a wavelength suitable for the fluorescent indicator; and detecting the fluorescent light from the sample.

**[0004]** In this case, the change of a signal (fluorescent light) from the cell or the tissue is generally remarkably small. Therefore, an intensity of the laser light with which the sample is irradiated is required to be stable at a high precision over a long time.

**[0005]** Some causes for which the intensity of the laser light for the irradiation of the sample is not stable are considered. There is a method of controlling the intensity of the laser light, comprising: monitoring the intensity of an emitted laser light; feeding the laser light intensity back to a controller; and controlling the laser light intensity. However, such feedback is not performed in a general helium neon laser. Therefore, an output power of the helium neon laser fluctuates with a change of environmental temperature, and the like.

**[0006]** Moreover, there is a multi-wavelength oscillation. For example, argon lasers oscillate with the laser light of wavelengths of 488 nm, 514.5 nm. Some of the argon lasers monitor and feedback-control the intensity of the emitted laser light.

**[0007]** However, the argon laser monitors a general output of the argon laser light of the wavelengths of 488 nm, 514.5 nm. The outputs of respective lines of these wavelengths compete among emission modes (wavelengths of 488 nm, 514.5 nm), and thereby each emission wavelength fluctuates. Furthermore, by consumption of an argon gas, an intensity ratio of the emission outputs of the argon laser light (intensity ratio of the wavelengths of 488 nm, 514.5 nm) changes with a use time.

**[0008]** On the other hand, there is a laser microscope for introducing the laser light into an optical fiber and guiding the laser light into a laser microscope main body by the optical fiber. In the laser microscope, the intensity of the laser light with which the sample is irradiated fluctuates by an output fluctuation by the optical fiber during undergoing of the change of environmental tempera-

ture, and a fluctuation of a light introduction efficiency by thermal deformation of a constituting element.

**[0009]** The intensity of the laser light fluctuates by the aforementioned causes, although the signal (fluorescent light) from the sample does not actually change. In this case, an erroneous result is possibly caused as if there were the change of the signal.

**[0010]** A technique for stabilizing the intensity of the laser light with which the sample is irradiated is disclosed, for example, in Jpn. Pat. Appln. KOKAI Publication Nos. 11-231222 and 2000-206415. In the Jpn. Pat. Appln. KOKAI Publication No. 11-231222, after the laser lights of a plurality of wavelengths are combined, some of the laser lights are split by a beam splitter. Subsequently, a changeable filter selects the wavelength, and an optical detector (first detection element) receives the laser light of the selected wavelength, and detects the intensity of the laser light of the wavelength. Moreover, a laser output or a laser intensity is controlled based on a detection signal of the laser light intensity. It is described that the laser intensity is controlled, for example, by an acousto-optical element (e.g., AOTF) disposed between the laser and the optical fiber.

**[0011]** The Jpn. Pat. Appln. KOKAI Publication No. 2000-206415 discloses a method comprising: controlling an operation in combination with a linear filter ring driven by a control unit, an area selection filter ring, or a filter slider; detecting an output of a selected laser line; driving the AOTF based on the detection signal; and stabilizing the output of the selected laser line, in order to constantly monitor laser radiation connected to a scanning module.

**[0012]** In recent years, in order to further pursue a function of the cell or the tissue, it has strongly been requested to simultaneously detect two or more types of samples (fluorescent light) from the sample, and analyze the function. For example, for fluorescent proteins of different wavelengths, such as a green fluorescent protein (GFP: a protein emitting a green fluorescent light) and a red fluorescent protein (RFP: a protein emitting a red fluorescent light), a gene is developed in the cell, and observed with time.

**[0013]** In this case, the laser light with which the sample is to be irradiated needs to have a wavelength optimum for these fluorescent proteins GFP, RFP. Additionally, both the laser lights of the two wavelengths need to have the light intensities stabilized.

**[0014]** However, in the techniques described in the two publications, only the intensity of the laser light of one wavelength is stabilized, and the laser lights of two or more wavelengths cannot simultaneously be controlled so as to stabilize the intensities of the laser lights. JP 11 271 636 discloses a laser microscope, irradiating a sample with a laser light constituted of a plurality of emission wavelengths through an objective lens, and detecting fluorescent light from the sample.

**[0015]** To solve the problem, an object of the present invention is to provide a laser microscope capable of si-

multaneously and steadily controlling an intensity of a laser light constituted of a plurality of wavelengths with which a sample is to be irradiated for each wavelength.

[0016] According to the present invention, there is provided a laser microscope, which irradiate a sample with a laser light constituted of a plurality of emission wavelengths through an objective lens, and detecting a fluorescent light from the sample, the laser microscope comprising: a spectral resolution section configured to spectrally resolve the laser light; a light receiving element array configured to receive the laser lights spectrally resolved by the spectral resolution section; and a controller configured to receive an output signal of the light receiving element array and controlling the laser light for each emission wavelength.

[0017] As described above in detail, according to the present invention, there can be provided the laser microscope capable of simultaneously and steadily controlling an intensity of the laser light constituted of a plurality of wavelengths with which the sample is to be irradiated for each wavelength.

[0018] This summary of the invention does not necessarily describe all necessary features so that the invention may also be a sub-combination of these described features.

[0019] The invention can be more fully understood from the following detailed description when taken in conjunction with the accompanying drawings, in which:

FIG. 1 is a constitution diagram showing a first embodiment of a scanning type laser microscope according to the present invention;

FIG. 2 is a constitution diagram showing a second embodiment of the scanning type laser microscope according to the present invention; and

FIG. 3 is a constitution diagram in a block in the second embodiment of the scanning type laser microscope according to the present invention.

[0020] A first embodiment of the present invention will be described hereinafter with reference to the drawings.

[0021] FIG. 1 is a constitution diagram of a laser scanning microscope. An argon laser 2 is fixed on a base 1. The argon laser 2 emits a laser light of two wavelengths of  $\lambda_1 = 488 \text{ nm}$  and  $\lambda_2 = 514.5 \text{ nm}$ .

[0022] A scanning unit 3 is disposed on an optical path of the laser light emitted from the argon laser 2. The scanning unit 3 constitutes a part of a laser microscope main body 4. The scanning unit 3 is a unit which scans the laser light on a sample S. Therefore, in this scanning unit 3, a beam splitter 5 is disposed on the optical path of the laser light, and an X-Y scanner 6 is disposed on a reflection optical path of the beam splitter 5.

[0023] The X-Y scanner 6 scans the laser light in an X direction set in the laser microscope, and a Y direction crossing at right angles to the X direction.

[0024] The sample S is a cell in which, for example, a GFP and a yellow fluorescent protein (YFP) are sub-

jected to gene development.

[0025] Moreover, a dichroic mirror 8 and mirror 9 are disposed in series via a mirror 7 on a transmission optical path (optical path of a direction in which the light is incident upon the beam splitter 5 from the X-Y scanner 6) of the beam splitter 5.

[0026] The dichroic mirror 8 splits a fluorescent light of two wavelengths  $\lambda_1'$ ,  $\lambda_2'$  emitted when the sample S is irradiated with the laser light of two wavelengths of 488 nm and 514.5 nm. That is, the dichroic mirror 8 has a function for reflecting the fluorescent light of one wavelength  $\lambda_1'$  and transmitting the fluorescent light of the other wavelength  $\lambda_2'$ .

[0027] A confocal lens 10a, confocal pinhole 11a, band pass filter 12a and optical detector 13a are disposed on the reflection optical path of the dichroic mirror 8.

[0028] A confocal lens 10b, confocal pinhole 11b, band pass filter 12b and optical detector 13b are disposed on the reflection optical path of the mirror 9 which is on the transmission optical path of the dichroic mirror 8.

[0029] A mirror 15 and prism for observation 16 are disposed on a laser optical path scanned by the X-Y scanner 6 via a pupil projection lens 14. Either one of the mirror 15 and prism for observation 16 is disposed on the optical path by a switching apparatus 17. An objective lens 19 is disposed on the reflection optical path of the mirror 15 via a tube lens 18. Additionally, when the prism for observation 16 is disposed in the optical path, the sample S can visually be observed through an eyepiece lens 20.

[0030] A beam splitter 21 is disposed in the scanning unit 3. The beam splitter 21 is disposed on the optical path of the laser light extending to the beam splitter 5 from an AOTF 25. The beam splitter 21 extracts a part of the laser light of two wavelengths of  $\lambda_1 = 488 \text{ nm}$  and  $\lambda_2 = 514.5 \text{ nm}$ . A prism 22 is disposed on the optical path of the extracted laser light.

[0031] This prism 22 spectrally resolves the laser light of two wavelengths  $\lambda_1 = 488 \text{ nm}$  and  $\lambda_2 = 514.5 \text{ nm}$ . That is, the prism 22 splits the laser light into two laser lights of wavelengths  $\lambda_1 = 488 \text{ nm}$  and  $\lambda_2 = 514.5 \text{ nm}$ .

[0032] A two-split photodiode 23 is disposed in a spectrum emission direction of the prism 22. The two-split photodiode 23 has a function of a light receiving element array for receiving the laser lights spectrally resolved by the prism 22. A split surface of the two-split photodiode 23 is disposed in the same direction as a direction in which spectrum is resolved.

[0033] A controller 24 inputs a detection signal outputted from the two-split photodiode 23, and outputs a control signal for controlling (wavelength selection control, amplitude control) the AOTF 25 fixed to an output end of the argon laser 2 based on the detection signal so that respective light intensities of both lines of wavelengths  $\lambda_1 = 488 \text{ nm}$  and  $\lambda_2 = 514.5 \text{ nm}$  become constant.

[0034] The AOTF 25 is fixed to the output end of the argon laser 2. The AOTF 25 receives the control signal outputted from the controller 24, selects the wavelength from two emission wavelengths  $\lambda 1 = 488$  nm and  $\lambda 2 = 514.5$  nm, and continuously controls an emission output.

[0035] An operation of the scanning type laser microscope constituted as described above will next be described.

[0036] The laser light of two wavelengths  $\lambda 1 = 488$  nm and  $\lambda 2 = 514.5$  nm is emitted/outputted from the argon laser 2. The laser light is transmitted through the AOTF 25, and incident upon the beam splitter 21. A part of the laser light is extracted, and incident upon the prism 22.

[0037] The prism 22 spectrally resolves the laser light into the laser lights of two wavelengths  $\lambda 1 = 488$  nm and  $\lambda 2 = 514.5$  nm. Each one of the spectrally resolved laser lights of two wavelengths is incident upon each split surface of the two-split photodiode 23 as the laser light of each line.

[0038] The two-split photodiode 23 receives each one of the laser lights spectrally resolved by the prism 22 via each split surface and outputs each detection signal.

[0039] In this case, when there are output fluctuations in the lines of laser lights of two wavelengths  $\lambda 1 = 488$  nm and  $\lambda 2 = 514.5$  nm emitted/outputted from the argon laser 2, the fluctuations of the light intensities of these lines are detected by the two-split photodiode 23.

[0040] The controller 24 inputs each detection signal outputted from the two-split photodiode 23, and outputs the control signal to the AOTF 25 based on the detection signal so that the respective light intensities of both lines of the wavelengths  $\lambda 1 = 488$  nm and  $\lambda 2 = 514.5$  nm become constant.

[0041] The AOTF 25 receives the control signal outputted from the controller 24, selects the wavelength from two emission wavelengths  $\lambda 1 = 488$  nm and  $\lambda 2 = 514.5$  nm, and continuously controls the emission output.

[0042] As a result, the respective light intensities of both lines of the wavelengths  $\lambda 1 = 488$  nm and  $\lambda 2 = 514.5$  nm become constant.

[0043] Additionally, in the observation of the sample S of the cell in which the GFP and YFP are subjected to gene development, the light intensities of both lines of wavelengths  $\lambda 1 = 488$  nm and  $\lambda 2 = 514.5$  nm are controlled to be constant at different wavelength values, respectively.

[0044] The laser light, controlled so that the respective light intensities of both lines of wavelengths  $\lambda 1 = 488$  nm and  $\lambda 2 = 514.5$  nm become constant, is reflected by the beam splitter 5 and scanned in an X-Y direction by the X-Y scanner 6.

[0045] The scanned laser light is transmitted through the pupil projection lens 14, reflected by the mirror 15, transmitted through the tube lens 18, forms a spot by the objective lens 19, and is scanned on the sample S.

[0046] Respective fluorescent lights of wavelengths

$\lambda 1'$  and  $\lambda 2'$  emitted from the sample S return in a direction opposite to a direction of the optical path of the laser light with which the sample S is irradiated. That is, each fluorescent light is passed through the tube lens 18, mirror 15, pupil projection lens 14, and X-Y scanner 6 from the objective lens 19, further transmitted through the beam splitter 5, reflected by the mirror 7, and incident upon the dichroic mirror 8.

[0047] In the fluorescent light of two wavelengths  $\lambda 1'$ ,  $\lambda 2'$ , the dichroic mirror 8 reflects one fluorescent light of wavelength  $\lambda 1'$  and transmits the other fluorescent light of wavelength  $\lambda 2'$ . The fluorescent light of the wavelength  $\lambda 1'$  reflected by the dichroic mirror 8 is passed through the confocal lens 10a, confocal pinhole 11a, and band pass filter 12a and incident upon the optical detector 13a.

[0048] Additionally, the fluorescent light of the wavelength  $\lambda 2'$  transmitted through the dichroic mirror 8 is passed through the confocal lens 10b, confocal pinhole 11b, and band pass filter 12b and incident upon the optical detector 13b.

[0049] These optical detectors 13a, 13b output respective fluorescent intensity signals. Therefore, when these fluorescent intensity signals are accumulated in synchronization with a drive signal of the X-Y scanner 6, images of respective fluorescent lights of two wavelengths  $\lambda 1'$ ,  $\lambda 2'$  are formed.

[0050] As described above, in the first embodiment, the beam splitter 21 extracts a part of the laser light of two wavelengths  $\lambda 1 = 488$  nm and  $\lambda 2 = 514.5$  nm, the prism 22 spectrally resolves the laser light of two wavelengths  $\lambda 1 = 488$  nm and  $\lambda 2 = 514.5$  nm, and the two-split photodiode 23 detects the intensities of two lines spectrally resolved in this manner. Moreover, the controller 24 controls the AOTF 25 fixed to the output end of the argon laser 2 based on the detection signal outputted from the two-split photodiode 23 so that the respective light intensities of both lines of wavelengths  $\lambda 1 = 488$  nm and  $\lambda 2 = 514.5$  nm become constant.

[0051] Thereby, the light intensities of both lines of wavelengths  $\lambda 1 = 488$  nm and  $\lambda 2 = 514.5$  nm can simultaneously and steadily be controlled to be constant. Therefore, for example, the cell in which the GFP and YFP are subjected to the gene development can be observed as the sample S over a long time with a high reliability. Furthermore, since the two-split photodiode 23 is used, the laser microscope can inexpensively be constituted.

[0052] A second embodiment of the present invention will next be described with reference to the drawings. Additionally, the same part as that of FIG. 1 is denoted with the same reference numeral, and detailed description thereof is omitted.

[0053] FIG. 2 is a constitution diagram of the laser scanning microscope. An argon laser 31, helium neon laser 32, and helium neon laser 33 are disposed in a base 30.

[0054] The argon laser 31 mainly emits/outputs a la-

ser light of three wavelengths  $\lambda_1 = 488$  nm,  $\lambda_2 = 514.5$  nm,  $\lambda_3 = 457.9$  nm.

[0055] The helium neon laser 32 emits/outputs the laser light of a wavelength  $\lambda_4 = 543$  nm.

[0056] The helium neon laser 33 emits/outputs the laser light of a wavelength  $\lambda_5 = 633$  nm.

[0057] For these lasers, a dichroic mirror 34 is disposed on an emission optical path of the argon laser 31, a dichroic mirror 35 is disposed on the emission optical path of the helium neon laser 32, and a mirror 36 is disposed on the emission optical path of the helium neon laser 33.

[0058] Therefore, the respective laser lights emitted/outputs from these lasers 31, 32, 33 are combined into one laser light by the dichroic mirrors 34, 35 and mirror 36.

[0059] Moreover, the AOTF 25 is fixed to the emission end of one laser light in the base 30. The AOTF 25 selects an arbitrary combination of wavelengths with respect to five emission wavelengths  $\lambda_1 = 488$  nm,  $\lambda_2 = 514.5$  nm,  $\lambda_3 = 457.9$  nm,  $\lambda_4 = 543$  nm,  $\lambda_5 = 633$  nm, and continuously controls the emission output by the control of the controller 24.

[0060] A fiber coupler 39 is fixed to the emission end of the AOTF 25. One end of an optical fiber 38 is fixed to the fiber coupler 39, and a converging lens 37 is disposed inside the coupler. One optical fiber end 38a of the optical fiber 38 is positioned in a converging position of the converging lens 37 in the fiber coupler 39.

[0061] On the other hand, one block 40 for resolving and monitoring the spectrum is detachably attached to the scanning unit 3 constituting the laser microscope main body 4. FIG. 3 is an enlarged constitution diagram of the block 40.

[0062] Other optical fiber end 38b of the optical fiber 38 is inserted/fixed into the block 40. A beam splitter 42 is disposed via a collimator lens 41 on the optical path of the laser light emitted from the optical fiber end 38b. The beam splitter 42 extracts a part of the laser light collimated by the collimator lens 41.

[0063] A prism 43 is disposed on the optical path of the laser light extracted by the beam splitter 42.

[0064] The prism 43 spectrally resolves the laser light emitted from the optical fiber end 38b into the laser lights of five wavelengths  $\lambda_1 = 488$  nm,  $\lambda_2 = 514.5$  nm,  $\lambda_3 = 457.9$  nm,  $\lambda_4 = 543$  nm,  $\lambda_5 = 633$  nm.

[0065] A one-dimensional CCD 45 is disposed via a converging lens 44 in a spectrum emission direction of the prism 43. The one-dimensional CCD 45 receives the laser lights spectrally resolved by the prism 43, and outputs each detection signal for each wavelength.

[0066] Concretely, the one-dimensional CCD 45 has five divided block surfaces corresponding to the respective lines of the wavelengths  $\lambda_1 = 488$  nm,  $\lambda_2 = 514.5$  nm,  $\lambda_3 = 457.9$  nm,  $\lambda_4 = 543$  nm,  $\lambda_5 = 633$  nm, and outputs each detection signal via each block surface.

[0067] Additionally, the detection signals indicate a sum of output signals of the respective elements for the

respective block surfaces.

[0068] The controller 24 inputs the respective detection signals outputted from the one-dimensional CCD 45, and outputs the control signal to the AOTF 25 based on the detection signals so that the respective light intensities of the respective lines of the wavelengths  $\lambda_1 = 488$  nm,  $\lambda_2 = 514.5$  nm,  $\lambda_3 = 457.9$  nm,  $\lambda_4 = 543$  nm,  $\lambda_5 = 633$  nm become constant.

[0069] Additionally, dichroic mirrors 8a, 8b, 8c, confocal lenses 10a, 10b, 10c, 10d, confocal pinholes 11a, 11b, 11c, 11d, band pass filters 12a, 12b, 12c, 12d, optical detectors 13a, 13b, 13c, 13d, and mirror 9 are disposed in the scanning unit 3, so that the sample S marked with four types of fluorescent lights at maximum can simultaneously be observed.

[0070] The operation of the laser scanning microscope constituted as described above will next be described.

[0071] The argon laser 31 emits/outputs the laser light of three wavelengths  $\lambda_1 = 488$  nm,  $\lambda_2 = 514.5$  nm,  $\lambda_3 = 457.9$  nm. The helium neon laser 32 emits/outputs the laser light of wavelength  $\lambda_4 = 543$  nm. The helium neon laser 33 emits/outputs the laser light of wavelength  $\lambda_5 = 633$  nm.

[0072] These laser lights are combined into one laser light by the dichroic mirrors 34, 35 and mirror 36.

[0073] The AOTF 25 selects the arbitrary combination of wavelengths from the combined laser light.

[0074] The laser light having the wavelength selected by the AOTF 25 is converged by the converging lens 37 and incident upon the one optical fiber end 38a of the optical fiber 38.

[0075] The laser light is propagated through the optical fiber 38 and emitted from the other optical fiber end 38b inserted into the block 40.

[0076] The laser light emitted from the optical fiber end 38b is collimated by the collimator lens 41, and a part of the laser light is extracted by the beam splitter 42 and incident upon the prism 43.

[0077] The prism 43 spectrally resolves the laser light emitted from the optical fiber end 38b into the laser lights of five wavelengths  $\lambda_1 = 488$  nm,  $\lambda_2 = 514.5$  nm,  $\lambda_3 = 457.9$  nm,  $\lambda_4 = 543$  nm,  $\lambda_5 = 633$  nm.

[0078] The laser lights of respective lines of wavelengths  $\lambda_1 = 488$  nm,  $\lambda_2 = 514.5$  nm,  $\lambda_3 = 457.9$  nm,  $\lambda_4 = 543$  nm,  $\lambda_5 = 633$  nm spectrally resolved in this manner are incident upon five block surfaces of the one-dimensional CCD 45.

[0079] The one-dimensional CCD 45 receives the laser lights spectrally resolved by the prism 43, and outputs each detection signal via each block surface.

[0080] In this case, when there is the output fluctuation in the laser light of the wavelength oscillated in the arbitrary combination of the respective lines of wavelengths  $\lambda_1 = 488$  nm,  $\lambda_2 = 514.5$  nm,  $\lambda_3 = 457.9$  nm,  $\lambda_4 = 543$  nm,  $\lambda_5 = 633$  nm, the one-dimensional CCD 45 detects the fluctuations of the light intensities of these lines and outputs the detection signals.

[0081] The controller 24 inputs the respective detection signals outputted from the one-dimensional CCD 45, and outputs the control signal to the AOTF 25 based on the detection signals so that the respective light intensities of respective lines of wavelengths  $\lambda_1 = 488$  nm,  $\lambda_2 = 514.5$  nm,  $\lambda_3 = 457.9$  nm,  $\lambda_4 = 543$  nm,  $\lambda_5 = 633$  nm become constant.

[0082] The AOTF 25 receives the control signal outputted from the controller 24, selects the wavelength with respect to five emission wavelengths  $\lambda_1 = 488$  nm,  $\lambda_2 = 514.5$  nm,  $\lambda_3 = 457.9$  nm,  $\lambda_4 = 543$  nm,  $\lambda_5 = 633$  nm, and continuously controls the emission output.

[0083] As a result, the respective light intensities of lines of wavelengths  $\lambda_1 = 488$  nm,  $\lambda_2 = 514.5$  nm,  $\lambda_3 = 457.9$  nm,  $\lambda_4 = 543$  nm,  $\lambda_5 = 633$  nm become constant.

[0084] The laser light, controlled so that the respective light intensities of lines of wavelengths become constant, is reflected by the beam splitter 5 and scanned in the X-Y direction by the X-Y scanner 6.

[0085] The scanned laser light is transmitted through the pupil projection lens 14, reflected by the mirror 15, transmitted through the image forming lens 18, forms the spot by the objective lens 19, and is scanned on the sample S.

[0086] Each fluorescent light emitted from the sample S returns in the direction opposite to the direction of the optical path of the laser light with which the sample S is irradiated. That is, each fluorescent light is passed through the tube lens 18, mirror 15, pupil projection lens 14, and X-Y scanner 6 from the objective lens 19, further transmitted through the beam splitter 5, reflected by the mirror 7, and incident upon the dichroic mirror 8a.

[0087] Then, by each of the dichroic mirrors 8a to 8c, the fluorescent lights of respective wavelengths are divided and are incident upon each of the optical detectors 13a, 13b, 13c, 13d. Then, these optical detectors 13a, 13b, 13c, 13d output respective fluorescent intensity signals. Thus, when these fluorescent intensity signals are accumulated in synchronization with the drive signal of the X-Y scanner 6, the respective fluorescent images of the sample S marked with four types of fluorescent lights at maximum are formed.

[0088] As described above, in the second embodiment, the beam splitter 42 extracts a part of the laser light of the arbitrary combination of wavelengths selected from the laser light of five wavelengths  $\lambda_1 = 488$  nm,  $\lambda_2 = 514.5$  nm,  $\lambda_3 = 457.9$  nm,  $\lambda_4 = 543$  nm,  $\lambda_5 = 633$  nm, the prism 43 spectrally resolves the laser light of these wavelengths, and the one-dimensional CCD 45 detects the respective intensities of the lines spectrally resolved in this manner. Moreover, the controller 24 controls the AOTF 25 based on the respective detection signals outputted from the one-dimensional CCD 45 so that the respective light intensities of the lines become constant.

[0089] Therefore, the light intensities of five lines of wavelengths  $\lambda_1 = 488$  nm,  $\lambda_2 = 514.5$  nm,  $\lambda_3 = 457.9$  nm,  $\lambda_4 = 543$  nm,  $\lambda_5 = 633$  nm can simultaneously and

steadily be controlled to be constant.

[0090] As a result, the gene developed cell of the sample S can be observed for a long time with a high reliability.

5 [0091] Moreover, the one-dimensional CCD 45 is used, so that the respective intensities of laser lines of a plurality of wavelengths can be controlled to be constant, and a design with a high degree of freedom is possible.

10 [0092] Furthermore, the collimator lens 41, beam splitter 42, prism 43, converging lens 44 and one-dimensional CCD 45 are constituted as one block 40. For example, there is another laser scanning microscope in which the scanning unit 3 is connected to the inverted type microscope main body. In this case, when erected and inverted types are to be selectively used in accordance with a purpose of use, a block including a laser light source and CCD for detecting the light intensity of each line can be used in common, and can inexpensively be constituted.

15 [0093] According to the present invention, there is provided a laser microscope for irradiating a samples with a laser light constituted of a plurality of oscillation wavelengths through an objective lens 19, and detecting a fluorescent light from the samples, the laser microscope comprising: an optical fiber 38 for guiding the laser light; a collimator lens 41 for collimating the laser light guided by the optical fiber 38; a beam splitter 42 for splitting a part of the laser light collimated by the collimator lens 41; a spectral resolution section 43 for spectrally resolving the laser light split by the beam splitter 42; a converging lens 44 for converging the laser light spectrally resolved by the spectral resolution section 43; a light receiving element array 45 for receiving the laser light converged by the converging lens 44; and a controller 24, 25 for receiving an output signal of the light receiving element array 45 and controlling the laser light for each oscillation wavelength.

20 [0094] Said collimator lens 41, said beam splitter 42, said spectral resolution section 43, said converging lens 44, and said light receiving element array 45 are formed into one block 40, and the block is constituted to be attachable/detachable with respect to a main body 4 of said laser microscope.

25 [0095] Additionally, the present invention is not limited to the first and second embodiments, and can variously be modified within the scope of the present invention in an implementation stage.

30 [0096] For example, the spectral resolution section in the first and second embodiments is not limited to the prisms 22, 43, and a diffraction grating or a beam splitter may also be used.

35 [0097] In order to control the respective laser light intensities of the plurality of wavelengths to be constant, a current value which emits each laser may independently be controlled, thereby constantly controlling the respective laser light intensities of the plurality of wavelengths.

[0098] Another control method may comprise disposing respective AOTFs in the argon lasers 2, 31, and helium neon lasers 32, 33, and controlling the respective laser light intensities of the plurality of wavelengths to be constant by these AOTFs disposed every laser.

### Claims

1. A laser microscope comprising an objective lens, a fluorescence detector (13a, 13b, 13c, 13d), and means for irradiating a sample by a laser light constituted of a plurality of emission wavelengths through said objective lens, and means for detecting fluorescent light emanating from the sample, **characterized by**:
  - a spectral resolution section (22, 43) configured to spectrally resolve said laser light by which the sample is irradiated;
  - a light receiving element array (23, 45) configured to receive the laser light spectrally resolved by the spectral resolution section
  - laser-light adjusting means (25) configured to adjust the intensity of the laser-light by which the sample is irradiated for each of the emission wavelengths; and
  - a controller (24) configured to control the laser-light adjusting means based on an output signal of the light receiving element array.
2. The laser microscope according to claim 1, **characterized in that** said spectral resolution section (22, 43) is any one selected from a group including of a prism, a diffraction grating, and a beam splitter.
3. The laser microscope according to claim 1, **characterized in that** said light receiving element array (23, 45) comprises either one of a split photodiode and a solid image pickup element.
4. The laser microscope according to claim 1, **characterized by** further comprising an optical fiber (38) for guiding said laser light into a laser microscope main body (4).
5. The laser microscope according to claim 1, **characterized by** further comprising an optical fiber (38) for guiding said laser light into a laser microscope main body (4), wherein said spectral resolution section (22, 43) and said light receiving element array (23, 45) are disposed on a light emission side of said optical fiber (38).
6. The laser microscope according to claim 1, **characterized in that** said controller (24) receives the output signal of said light receiving element array (23, 45) and simultaneously controls respective light intensities of the plurality of emission wavelengths of said laser light to be constant.
7. The laser microscope according to claim 1, **characterized in that** said controller (24) comprises:
  - 10 a control unit (24) configured to receive the output signal of said light receiving element array (23, 45) and outputting a control signal for simultaneously setting respective light intensities of the plurality of emission wavelengths of said laser light to be constant; and
  - 15 an acousto-optical element (25), disposed on an optical path of said laser light, configured to receive said control signal outputted from said control unit (24) and setting the respective light intensities of the plurality of emission wavelengths of said laser light to be constant.
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8. The laser microscope according to claim 1, **characterized in that** a converging lens (44) disposed between said spectral resolution section (43) and said light receiving element array (45) and configured to converge the spectrally resolved laser lights on said light receiving element array for the respective emission wavelengths.
9. The laser microscope according to claim 1, **characterized by** further comprising a beam splitter (21, 42) configured to split a part of said laser light and guiding the part into said spectral resolution section (22, 43).
10. The laser microscope according to claim 1, **characterized by** further comprising:
  - an output fiber (38) configured to guide said laser light;
  - a collimator lens (41) configured to collimate said laser light guided by the optical fiber; and
  - a beam splitter (42) configured to split a part of said laser light collimated by the collimator lens;
  - a converging lens (44) configured to converge the laser light spectrally resolved by the spectral resolution section.
11. The laser microscope according to claim 10, **characterized in that** said collimator lens (41), said beam splitter (42), said spectral resolution section (43), said converging lens (44), and said light receiving element array (45) are formed into one block (40), and the block is constituted to be attachable/detachable with respect to a main body (4) of said laser microscope.

## Patentansprüche

1. Lasermikroskop mit einer Objektivlinse, einem Fluoreszenzdetektor (13a, 13b, 13c, 13d) und einem Mittel zur Bestrahlung durch die Objektivlinse einer Probe mit einem Laserlicht, das aus einer Mehrzahl von Emissionswellenlängen gebildet ist, und mit einem Mittel zur Erfassung eines von der Probe ausgesendeten Fluoreszenzlichts, **gekennzeichnet durch:**
  - einen Abschnitt (22, 43) zur spektralen Auflösung, der ausgelegt ist, um das Laserlicht, mit dem die Probe bestrahlt wird, spektral aufzulösen;
  - ein Lichtempfangselementefeld (23, 45), das ausgelegt ist, um das **durch** den Abschnitt zur spektralen Auflösung spektral aufgelöste Laserlicht zu empfangen;
  - ein Laserlichteinstellungsmittel (25), das ausgelegt ist, um die Intensität des Laserlichts, mit dem die Probe bestrahlt wird, für jede Wellenlänge einzustellen; und
  - ein Regelungsgerät (24), das ausgelegt ist, um das Laserlichteinstellungsmittel auf der Grundlage eines Ausgangssignals des Lichtempfangselementefeldes einzustellen.
2. Lasermikroskop nach Anspruch 1, **dadurch gekennzeichnet, dass** der Abschnitt (22, 43) zur spektralen Auflösung ein aus einer Gruppe, die ein Prisma, ein Beugungsgitter und einen Strahlteiler enthält, ausgewählter Abschnitt ist.
3. Lasermikroskop nach Anspruch 1, **dadurch gekennzeichnet, dass** das Lichtempfangselementefeld (23, 45) entweder eine geteilte Photodiode oder ein festes Bildaufnahmeelement umfasst.
4. Lasermikroskop nach Anspruch 1, **dadurch gekennzeichnet, dass** es ferner eine Lichtleitfaser (38) zur Führung des Laserlichts in einen Lasermikroskop-Hauptkörper (4) umfasst.
5. Lasermikroskop nach Anspruch 1, **dadurch gekennzeichnet, dass** es ferner eine Lichtleitfaser (38) zur Führung des Laserlichts in einen Lasermikroskop-Hauptkörper (4) umfasst, wobei der Abschnitt (22, 43) zur spektralen Auflösung und das Lichtempfangselementefeld (23, 45) auf einer Lichtemissionsseite der Lichtleitfaser (38) angeordnet sind.
6. Lasermikroskop nach Anspruch 1, **dadurch gekennzeichnet, dass** das Regelungsgerät (24) das

5 Ausgangssignal von dem Lichtempfangselementefeld (23, 45) empfängt und gleichzeitig jeweilige Lichtintensitäten der Mehrzahl von Emissionswellenlängen des Laserlichts so regelt, dass sie konstant sind.

7. Lasermikroskop nach Anspruch 1, **dadurch gekennzeichnet, dass** das Regelungsgerät (24) umfasst:
  - eine Regelungseinheit (24), die ausgelegt ist, um das Ausgangssignal des Lichtempfangselementefeldes (23, 45) zu empfangen und ein Steuersignal auszugeben, um jeweilige Lichtintensitäten der Mehrzahl von Emissionswellenlängen des Laserlichts so einzustellen, dass sie konstant sind; und
  - ein akusto-optisches Element (25), das in einem Lichtweg des Laserlichts angeordnet ist und so ausgelegt ist, dass es das von der Regelungseinheit (24) ausgegebene Steuersignal empfängt und die jeweiligen Lichtintensitäten der Mehrzahl von Emissionswellenlängen des Laserlichts so einstellt, dass sie konstant sind.
8. Lasermikroskop nach Anspruch 1, **dadurch gekennzeichnet, dass** eine Sammellinse (44) zwischen dem Spektralauflösungsabschnitt (43) und dem Lichtempfangselementeabschnitt (45) angeordnet und ausgelegt ist, um die spektral aufgelösten Laserlichter auf dem Lichtempfangselementefeld für jeweilige Emissionwellenlängen umzukehren.
9. Lasermikroskop nach Anspruch 1, **dadurch gekennzeichnet, dass** es ferner einen Strahlteiler (21, 42) umfasst, der so ausgelegt ist, dass er einen Teil des Laserlichts abspaltet und den Teil in den Abschnitt (22, 43) zur spektralen Auflösung leitet.
10. Lasermikroskop nach Anspruch 1, **dadurch gekennzeichnet, dass** es ferner umfasst:
  - eine Auskopplungslichtleitfaser (38), die ausgelegt ist, um das Laserlicht zu führen;
  - eine Kollimatorlinse (41), die ausgelegt ist, um das durch die Lichtleitfaser geführte Laserlicht zu kollimieren; und
  - einen Strahlteiler (42), der ausgelegt ist, um einen Teil des durch die Kollimatorlinse kollimierten Laserlichts abzuspalten;
  - eine Sammellinse (44), die ausgelegt ist, um das durch den Spektralauflösungsabschnitt spektral aufgelöste Laserlicht zu bündeln.
11. Lasermikroskop nach Anspruch 10, **dadurch gekennzeichnet, dass** die Kollimatorlinse (41), der Strahlteiler (42), der Abschnitt (43) zur spektralen

Auflösung, die Sammellinse (44) und das Lichtempfangselementfeld (45) in einem Block (40) ausgebildet sind, und der Block ist so ausgebildet, dass er an dem Hauptkörper (4) des Lasermikroskops befestigt und von diesem getrennt werden kann.

### Revendications

1. Microscope laser incluant une lentille d'objectif, un détecteur de fluorescence (13a, 13b, 13c, 13d), un moyen destiné à irradier un échantillon à l'aide d'une lumière laser constituée d'une pluralité de longueurs d'ondes d'émission à travers ladite lentille d'objectif, et un moyen destiné à détecter une lumière fluorescente émanant de l'échantillon, **caractérisé par :**
  - une section de définition spectrale (22, 43) configurée pour définir de manière spectrale ladite lumière laser qui irradie l'échantillon ;
  - un groupement d'éléments de réception de lumière (23, 45) configuré pour recevoir la lumière laser définie de manière spectrale par la section de définition spectrale ;
  - un moyen de réglage de lumière laser (25) configuré pour régler l'intensité de la lumière laser qui irradie l'échantillon pour chacune des longueurs d'ondes d'émission ; et
  - un régisseur (24) configuré pour commander le moyen de réglage de lumière laser sur la base d'un signal de sortie du groupement d'éléments de réception de lumière laser.
2. Microscope laser selon la revendication 1, **caractérisé en ce que** ladite section de définition spectrale (22, 43) est constituée de l'un quelconque choisi d'un groupe incluant un prisme, un réseau de diffraction, et un diviseur de faisceau.
3. Microscope laser selon la revendication 1, **caractérisé en ce que** ledit groupement d'éléments de réception de lumière (23, 45) comprend soit une photodiode divisée soit un élément de saisie d'image d'une seule pièce.
4. Microscope laser selon la revendication 1, **caractérisé par le fait qu'il comprend en outre** une fibre optique (38) destinée à guider ladite lumière laser dans un corps principal (4) de microscope laser.
5. Microscope laser selon la revendication 1, **caractérisé par le fait qu'il comprend en outre** une fibre optique (38) destinée à guider ladite lumière laser dans un corps principal (4) de microscope laser, dans lequel ladite section de définition spectrale (22, 43) et ledit groupement d'éléments de réception de lumière (23, 45) sont disposés d'un côté
6. Microscope laser selon la revendication 1, **caractérisé en ce que** ledit régisseur (24) reçoit le signal de sortie dudit groupement d'éléments de réception de lumière (23, 45) et, simultanément, commande des intensités de lumière respectives de la pluralité de longueurs d'ondes d'émission de ladite lumière laser pour qu'elles soient constantes.
7. Microscope laser selon la revendication 1, **caractérisé en ce que** ledit régisseur (24) comprend :
  - un module de commande (24) configuré pour recevoir le signal de sortie dudit groupement d'éléments de réception de lumière (23, 45) et pour délivrer un signal de commande servant à fixer simultanément des intensités de lumière respectives de la pluralité de longueurs d'ondes d'émission de ladite lumière laser pour qu'elles soient constantes ; et
  - un élément acousto-optique (25), disposé sur un trajet optique de ladite lumière laser, configuré pour recevoir ledit signal de commande délivré par ledit module de commande (24) et pour fixer les intensités de lumière respectives de la pluralité de longueurs d'ondes d'émission de ladite lumière laser pour qu'elles soient constantes.
8. Microscope laser selon la revendication 1, **caractérisé en ce qu'une lentille convergente (44) est disposée entre** ladite section de définition spectrale (43) et ledit groupement d'éléments de réception de lumière (45) et est configurée pour faire converger les lumières laser définies de manière spectrale des longueurs d'ondes d'émission respectives sur ledit groupement d'éléments de réception de lumière.
9. Microscope laser selon la revendication 1, **caractérisé par le fait qu'il comprend en outre** un diviseur de faisceau (21, 42) configuré pour diviser une partie de ladite lumière laser et pour guider la partie dans ladite section de définition spectrale (22, 43).
10. Microscope laser selon la revendication 1, **caractérisé par le fait qu'il comprend en outre :**
  - une fibre de sortie (38) configurée pour guider ladite lumière laser ;
  - une lentille de collimation (41) configurée pour collimater ladite lumière laser guidée par la fibre optique ; et
  - un diviseur de faisceau (42) configuré pour diviser une partie de ladite lumière laser collimée par la lentille de collimation ;
  - une lentille convergente (44) configurée pour faire converger la lumière laser définie de ma-

nière spectrale par la section de définition spectrale.

11. Microscope laser selon la revendication 10, caractérisé en ce que ladite lentille de collimation (41),<sup>5</sup> ledit diviseur de faisceau (42), ladite section de définition spectrale (43), ladite lentille convergente (44) et ledit groupement d'éléments de réception de lumière (45) sont formés en un bloc (40), et en ce que le bloc est structuré pour pouvoir être assujetti<sup>10</sup> à un corps principal (4) dudit microscope laser et désolidarisé de celui-ci.

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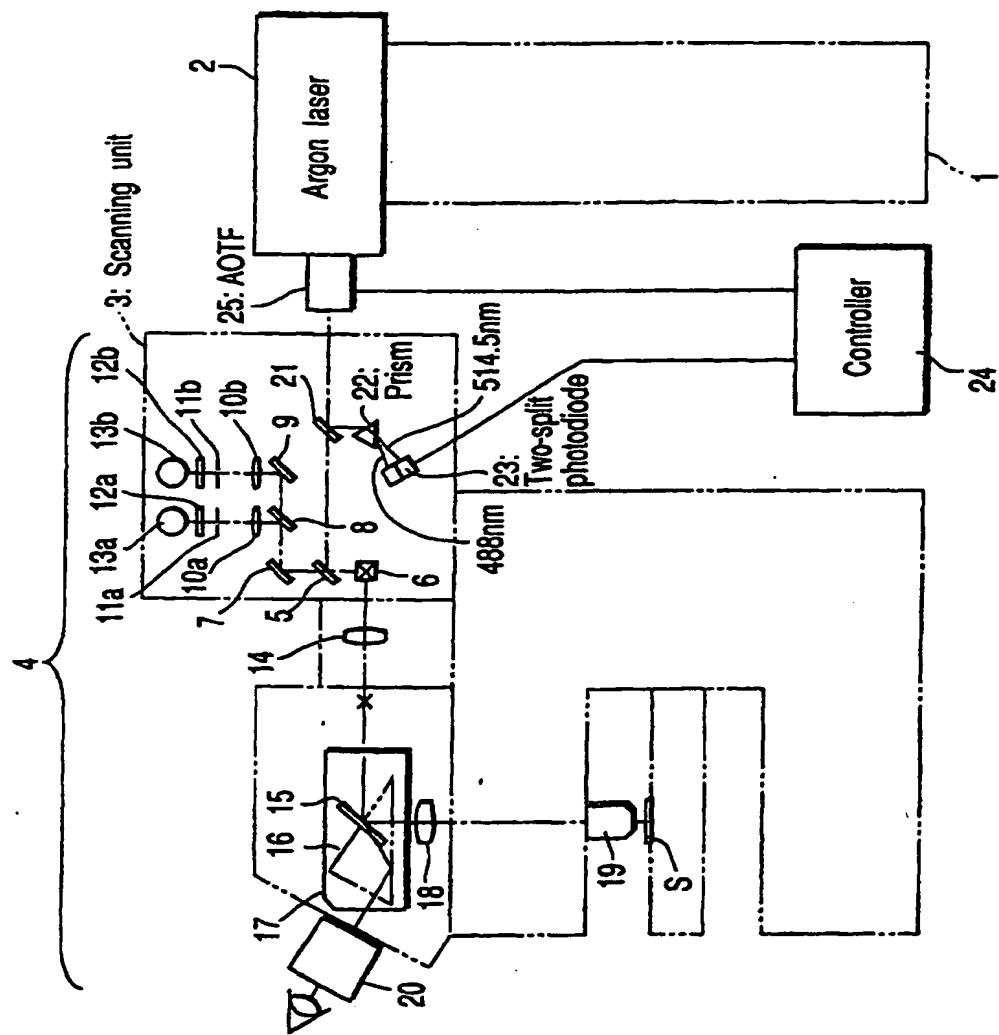


FIG. 1

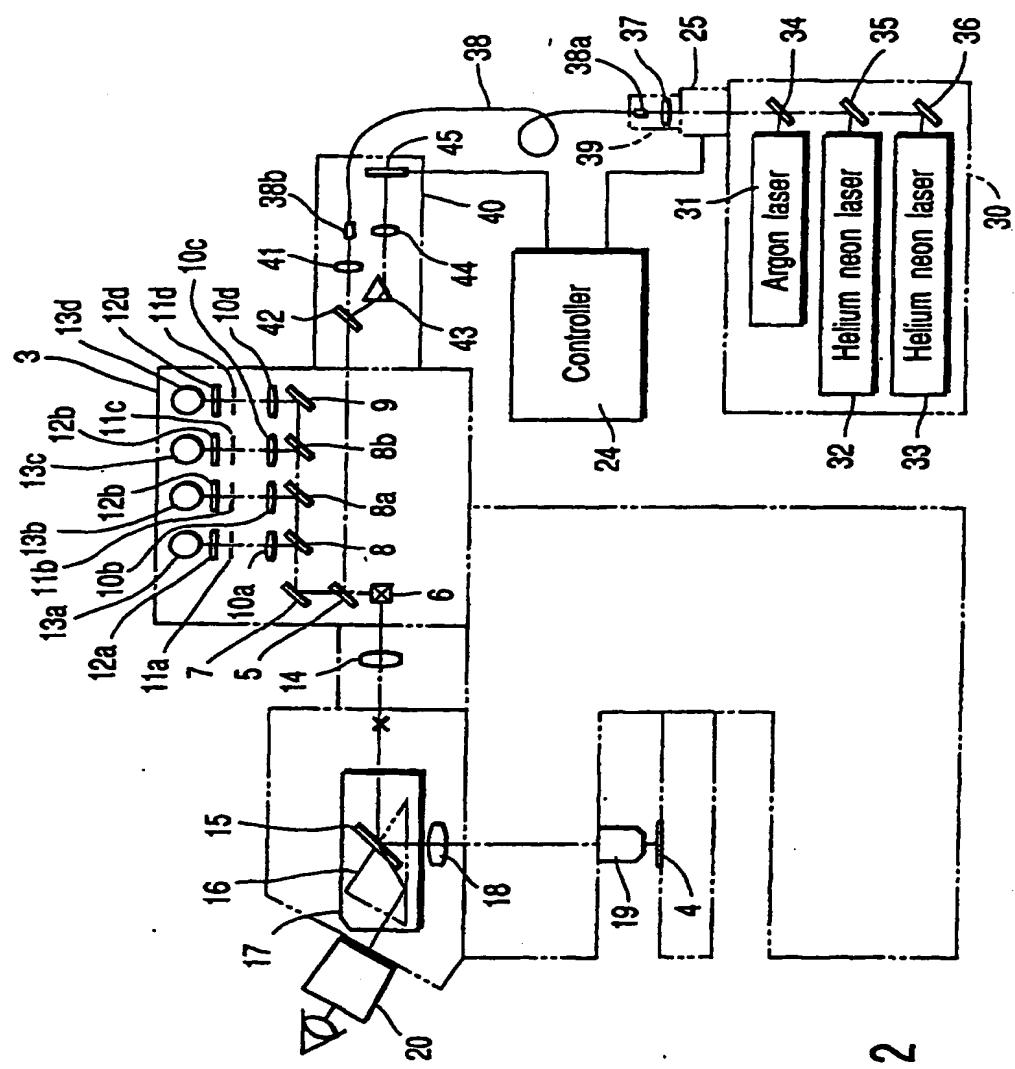


FIG. 2

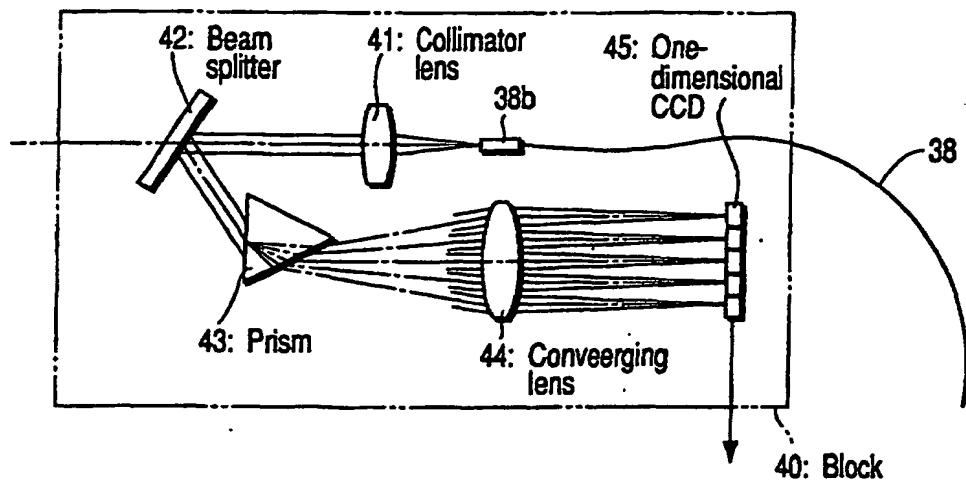


FIG. 3